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Selections of minimal conditions for a simple intensification and scale up of ω-transaminase reactions

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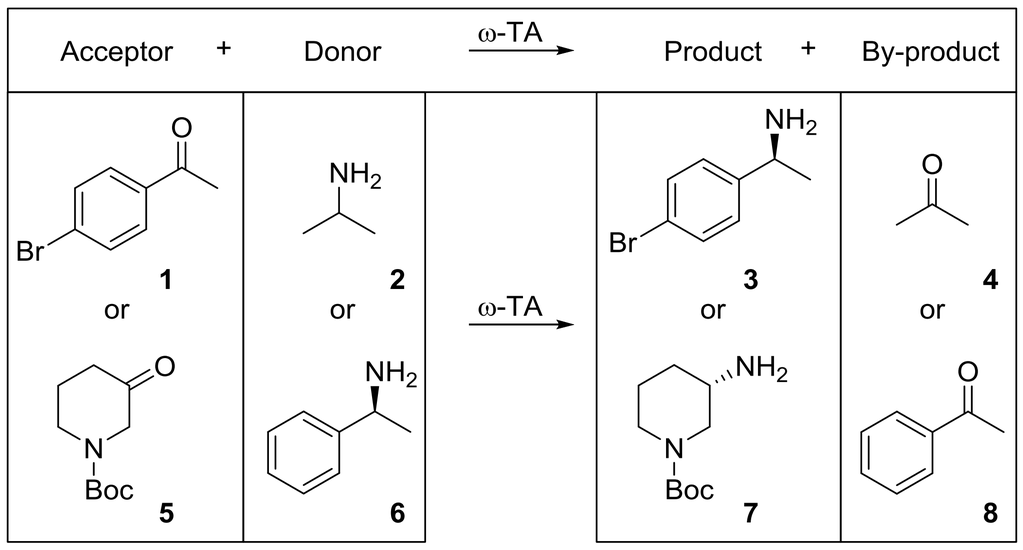
Introduction

A step wise decision matrix is presented to quickly evaluate ω-transaminase for a 'simple scale up' in the synthetic direction. Here a 'simple scale up' is defined as a system without specialized equipment or process development, thus a rapid implementation. The three step method consists of: 1. thermodynamic evaluation, 2. biocatalyst screen and 3. inhibition characterization. Each step of the method has a cut off value for easy implementation. Demonstrated by a case study which eliminated reaction pair candidates based on the cut off criteria. Finally, the most promising candidate was intensified.

Case study



1. Thermodynamics?

$K_{eq} > 1$

K_{eq}	Donor	
Acceptor	**2**	**6**
1	0.025	0.5
5	32	450

2. Biocatalyst Activity?

> 0.05 g/g/hr

Enzyme	g/g/hr[a]
Ars-ωTA	0.048
Tar0	0.003
Tar1	0.012
ATA 47	0.054

3. Enzyme Inhibition?

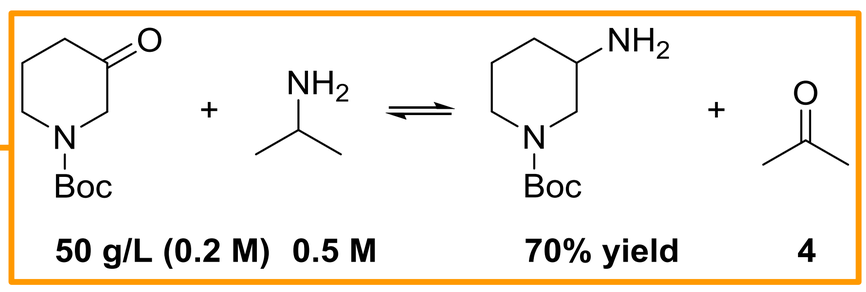
< 50% loss at 10mM [Prod]

Reactant	Activity[b]
5+2	90%
5+6	110%

a) Carried out with **5**+**2**
b) Initial activity with 10mM product present

Intensification

-Meets criteria



1. More than 20 fold excess of the donor is required for 95% conversion
2. Gives 95% yield in 100hr with a 50 g/L substrate
3. At higher substrate concentrations the reaction is assumed to significantly slow down

Conclusion

We have here outlined and successfully demonstrated a simple method for selecting reaction candidates suitable for a simple and rapid intensification and scale up. This can be valuable is processes where time is of the essence, such as small singular batches and pharmaceutical applications. Although, the ω-transaminase reaction is used here, we nonetheless argue that this method can be applied for other biocatalytic reactions, for well developed enzymes, where the limitations of the implementation is known.

Intensification

The best reaction candidate pair 1-Boc-3-piperidone and isopropylamine was successfully and intensified up to 75 g/L and ran at a 25 mL scale. The best isolated product yields were obtained with the 50 g/L reaction where 70% (S)-(+)-3-amino-1-Boc-piperidine was isolated.

DR. REDDY'S